TITLE: Studies of HmuT protein in the heme uptake pathway of Corynebacterium diphtheriae

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INTRODUCTION: Corynebacterium diphtheriae is the causative organism of the severe upper respiratory tract disease diphtheria. (Courtni et al., 2011). The human pathogen C. diphtheriae utilizes hemin and hemoglobin as iron sources for growth in iron-depleted environments (Courtni et al., 2011). The use of hemin iron in C. diphtheriae involves the iron-regulated hmu hemin, which is a heme-binding component (Courtni et al., 2011). This is being studied to understand which amino acids account for heme uptake and the process by which heme is taken up.

PURPOSE: The goal of this work was to purify Y235A and Y272A mutants of HmuT and to study the unfolding of these proteins to understand how tightly HmuT binds to heme. The long term goal is to understand the heme uptake pathway, which may lead to finding new therapeutic approaches to drug resistant bacteria.

METHOD: HmuT mutants with a strep-tag (WSHPNFGK) were grown in Luria-Bertini (LB) with kanamycin. The cells were incubated for 16 h and induced with isopropyl β-D-1-thiogalactopyranoside (IPTG). The cells were harvested by centrifugation and lysed, on ice, by sonication. The supernatant was collected and purified by FPLC. Myoglobin was used as a model protein to test the unfolding patterns before the denaturation of the expressed Y272A and Y235A mutants. Myoglobin was denatured using guanidine hydrochloride (GdnHCl). The unfolding of myoglobin was viewed via optical spectroscopy at room temperature. The curve was analyzed using the following equations:

\[
y = \frac{(yF + mF[D]) + (yU + mU[D] * \exp [m * ([D] - [D]_{1/2})/RT])}{(1 + \exp[m * ([D] - [D]_{1/2})/RT])}
\]

\[
((m1+m2*m0)+(m3+m4*m0)*\EXP(m5*(m0-m6)/(1.987*295)))/(1+\EXP(m5*(m0-
m6)/(1.987*295)))
\]

The data of the unfolding curve of myoglobin was fitted in Kaleidagraph using the unfolding equation above.

RESULT: As a result, the yield of heme bound protein was 3.0 mg/ml for Y272A. Its spectrum is consistent with the histidine/tyrosine axial ligand pair. The yield of heme bound protein was 1.1 mg/ml for Y235A. Its spectrum is consistent with the histidine axial ligand. Myoglobin was successfully denatured. The D_{1/2} = 1.06 ± 0.04, which is very similar to literature values.
CONCLUSION: In all, the mutants of HmuT were expressed and purified. The spectra for the mutants show their respective invariable ligand pairs. Myoglobin was used to study unfolding patterns. The results are comparable to the literature value found.